# Consolidated Microbial Commercial Activity Notice (MCAN)

# PC7230

## Contained Use of Genetically Engineered Strains of

## *Saccharomyces cerevisiae*

## TS-AEH507

March 17, 2015

Submitted by

Taurus Energy AB

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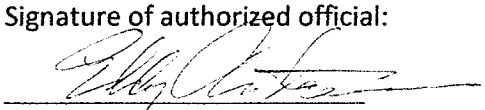
**CERTIFICATION STATEMENT**

I certify that to the best of my knowledge and belief: The company named in this submission intends to manufacture, import, or process for a commercial purpose, other than in small quantities solely for research and development, the microorganism identified in this submission. All information provided in this submission is complete and truthful as of the date of submission. I am including with this submission all test data in my possession or control and a description of all other data known to or reasonably ascertainable by me as required by 40 CFR 725.160 or 725.260.

The company identified in this notice is a small business concern under 40 CFR 700.43 and has remitted a fee of $100 in accordance with 40 CFR 700.45(d).

Signature of authorized official:



Name: Eddy Christensson

Title: Vice President, Marketing and Sales, North America

Date: March 17, 2015

**CONFIDENTIAL BUSINESS CLAIMS**

C. General questions

The following questions must be answered in detail for each confidentiality claim:

1. For what period of time is a claim of confidentiality being asserted? If the claim is to extend until a certain event or point in time, indicate that event or time period. Explain why the information should remain confidential until such point.

The information is claimed confidential permanently, or until Applicant makes the information common knowledge. Disclosure of the information would put Applicant at a substantial technology disadvantage with respect to potential competitors.

2. Briefly describe any physical or procedural restrictions within the company or institution relating to the use and storage of the information claimed as confidential. What other steps, if any, apply to use or further disclosure of the information?

Documents containing the information claimed as confidential are marked Company Confidential. Disclosure of the information within the Company is on a need-to-know basis and all Applicant employees have signed employment contracts that include strict confidentiality provisions, including a prohibition on unauthorized disclosure of information such as the information claimed as Confidential herein.

3. Has the information claimed as confidential been disclosed to individuals outside of the company or institution? Will it be disclosed to such persons in the future? If so, what restrictions, if any, apply to use or further disclosure of the information?

Certain of the information has been, or may be, disclosed to people or companies that have entered into, or that are discussing, business relationships with Applicant. Any individual or company outside of Applicant who needs to know the claimed CBI in the course of their business with the Company must, before receiving any such information, sign a written nondisclosure agreement to hold the information confidential and proprietary to the Company.

4. Does the information claimed as confidential appear, or is it referred to, in any of the following questions. If the answer is yes to any of these questions, indicate where the information appears and explain why it should nonetheless be treated as confidential.

(1) Advertising or promotional materials for the microorganism or the resulting end product.

At this time, Applicant does not have any advertising or promotional material, but any such materials it develops in the future will not contain any information claimed as confidential in this MCAN.

(2) Material safety data sheets or other similar materials for the microorganism or the resulting end product.

At this time, Applicant has not created material safety data sheets or similar materials for the MCAN strains, but any such materials it develops in the future will not contain any information claimed as confidential in this MCAN.

(3) Professional or trade publications.

No. While Applicant may encourage its scientists and engineers to publish in professional or trade journals, we expect that such publications would only disclose select elements of strain construction, biological performance, or process design. The complete information about Applicant's commercial production strains and our process technology is proprietary and would not be permitted to be published.

(4) Any other media available to the public or to your competitors.

No. Any individual or company outside of Applicant who needs to know the claimed CBI in the course of their business with the Company must, before receiving any such information, sign a written nondisclosure agreement to hold the information confidential and proprietary to the Company.

(5) Patents.

No. While some elements of strain construction or process design claimed hereunder as confidential are or might be the subject of issued or pending patent applications, the complete information about Applicant's commercial production strains and process technology are proprietary and not fully disclosed in any of the company's pending or issued patents.

(6) Local, State, or Federal agency public files.

No, to the best of our knowledge, confidential information is not disclosed in these public files. Any information which may have been provided to any such agency would have been claimed and labeled as "Confidential".

5. Has EPA, another Federal agency, a Federal court, or a State made any confidentiality determination regarding the information claimed as confidential? If so, provide copies of such determinations.

No.

6. For each type of information claimed confidential, describe the harm to the company or institution's competitive position that would result if this information were disclosed. Why would this harm be substantial? How could a competitor use such information? What is the causal connection between the disclosure and harm?

Although some general aspects of Applicant's strategies for genome modification are known to the public, the specific identities and sequences of the genes being modified and the regulatory sequences being used are not known and have not been disclosed to the public. Disclosure of such information, particularly the genetic sequences, would give Applicant's competitors a significant advantage in allowing them to recreate (i.e. using DNA synthesis) some or all of Applicant's proprietary genes. Similarly, Applicant has invested substantial time and effort in the design of proprietary methods for the

optimal growth of our production organisms, and disclosure of such information would allow our competitors to duplicate and use the same or similar conditions. In either case, disclosure of our confidential information could allow competitors to create products or processes similar to, and in competition with, Applicant's, without incurring the cost and expense that Applicant has incurred in our R&D.

7. If EPA disclosed to the public the information claimed as confidential, how difficult would it be for the competitor to enter the market for the resulting product? Consider such constraints as capital and marketing cost, specialized technical expertise, or unusual processes.

Disclosure of Applicant's biological information would provide an opportunity for any competitor skilled in the art of recombinant DNA technology to easily recreate the genetic constructs used to create Applicant's production microorganisms, thereby capitalizing on Applicant's research and development efforts and matching our product's performance in a short time period with minimal cost or capital investment.

D. Microorganism identity and production method.

If confidentiality claims are asserted for the specific identity of the microorganism or information on how the microorganism is produced, the following questions must be answered:

1. Has the microorganism or method of production been patented in the U.S. or elsewhere? If so, why is confidentiality necessary?

While some elements of strain construction claimed hereunder as confidential are or might be the subject of pending patent applications, the complete information about Applicant's commercial production strains is proprietary and is not fully disclosed in any of the company's pending or issued patents. Further, our patents generally disclose and claim many different genes and constructs, and so it is not readily apparent from the patent filings which genes and constructs would be used in a the commercial product or process.

2. Does the microorganism leave the site of production or testing in a form which is accessible to the public or to competitors? What is the cost to a competitor, in time and money, to develop appropriate use conditions? What factors facilitate or impede product analysis?

No. The microorganism does not leave the site of production in a form accessible to the public or competitors. The microorganism is not present in the final commercial product. If a competitor either had access to the subject organism, or could reconstruct it through publicly disclosed information, it would be relatively straightforward for one skilled in fermentation to develop appropriate use conditions (less than one year effort) in order to commercialize a competitive offering.

## Submitter identification

a) Submitter

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## A. RECIPIENT ORGANISM CHARACTERIZATION

### A.1. Taxonomy: General

This is a consolidated MCAN covering 5 modified strains based on the recipient organism *Saccharomyces cerevisiae*. The general taxonomy of these strains is as follows:

| | |
|---|---|
| Kingdom: | Fungi |
| Phylum: | Ascomycota |
| Subphylum: | Saccharomycotina |
| Class: | Saccharomycetes |
| Order: | Saccharomycetales |
| Family: | Saccharomycetaceae |
| Genus: | Saccharomyces |
| Species: | cerevisiae |

### A.2. Taxonomy: Specific issues.

The recipient strain for the MCAN microorganisms is *Saccharomyces cerevisiae* strain [redacted]

[redacted]

## B. SUBJECT ORGANISM CHARACTERIZATION

### B.1. The Subject Microorganism.

#### *B.1.a. Overview.*

The generic name for the MCAN microorganisms is "Modified *Saccharomyces cerevisiae* for the production of ethanol."

#### *B.1.b. Summary of Genetic Modifications.*

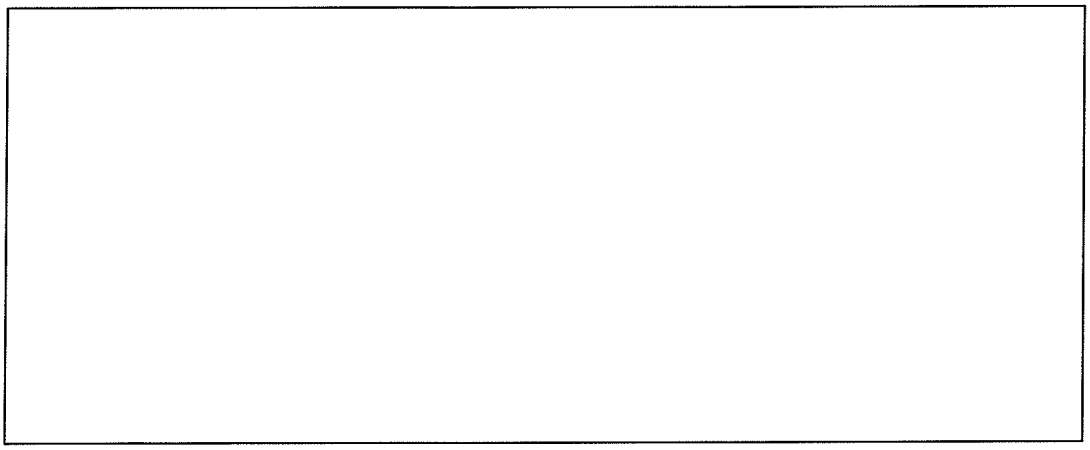
**Table B-1** summarizes the genetic changes introduced into *S. cerevisiae* .

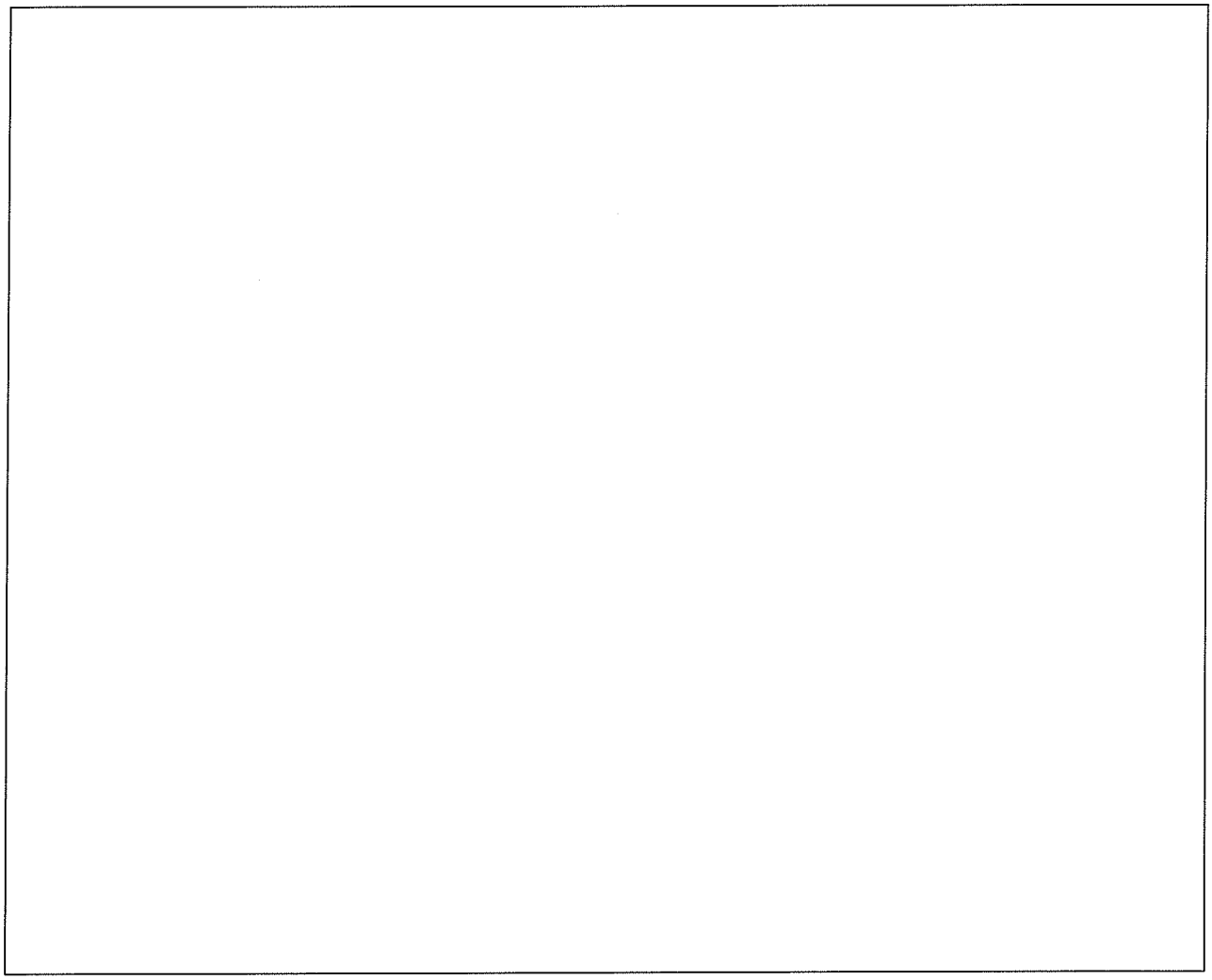
**Table B-1. Genetic changes in MCAN strains.**

***B.1.c. Taxonomy of Donor Microorganism.***

**B.2. Constructs used for Integration**

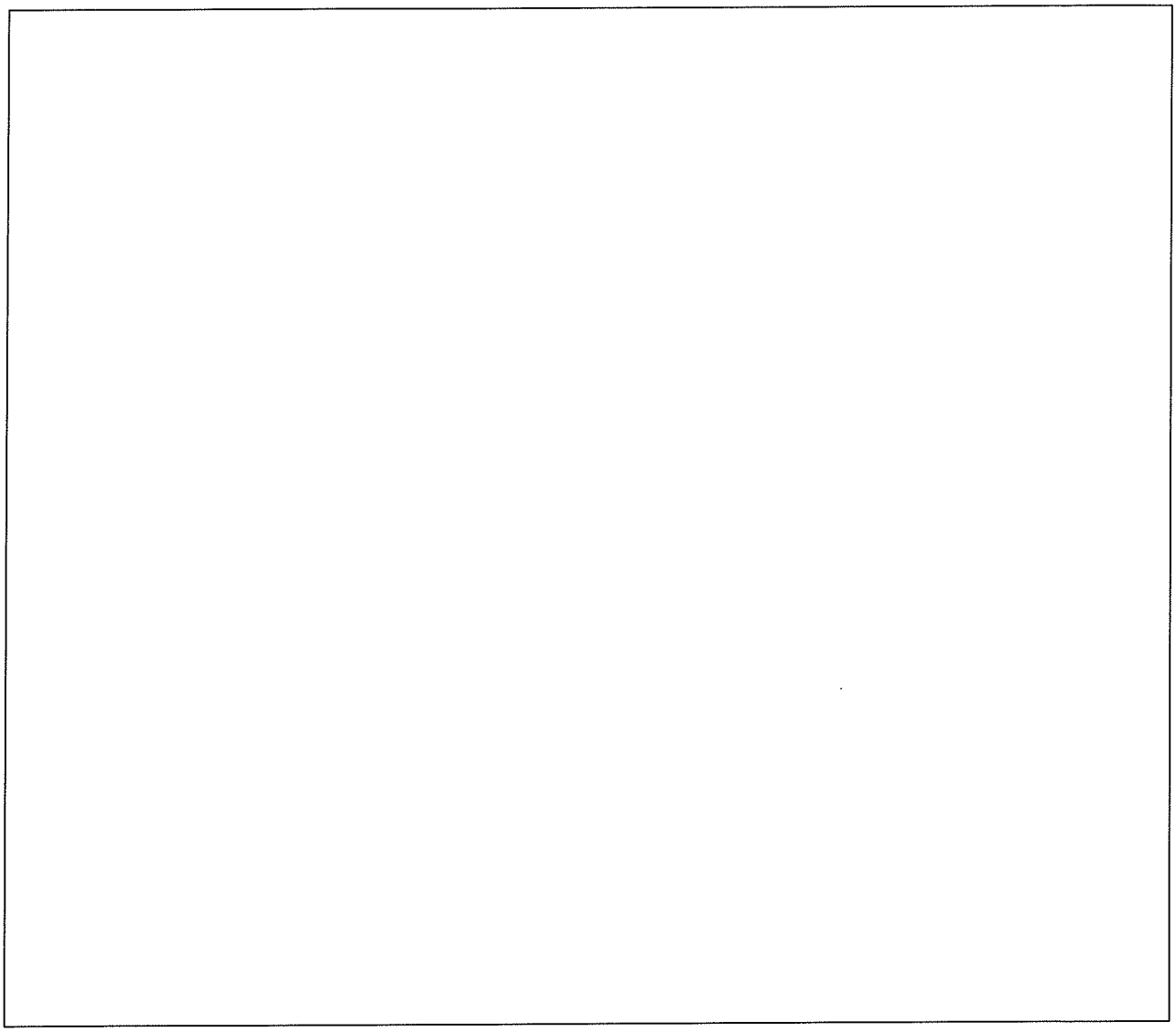
**Figure B-1. Constructs for Integration.**

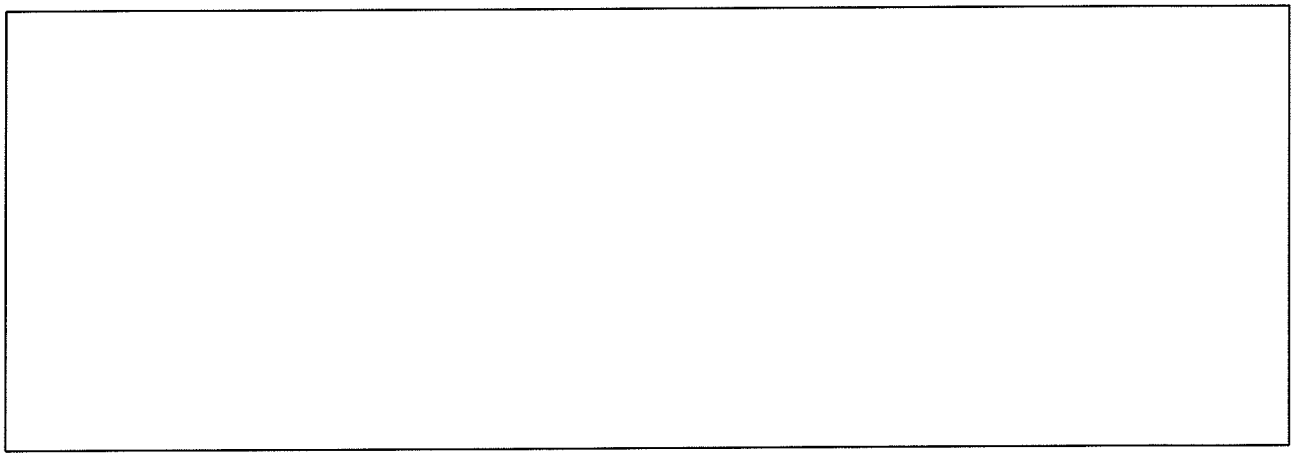


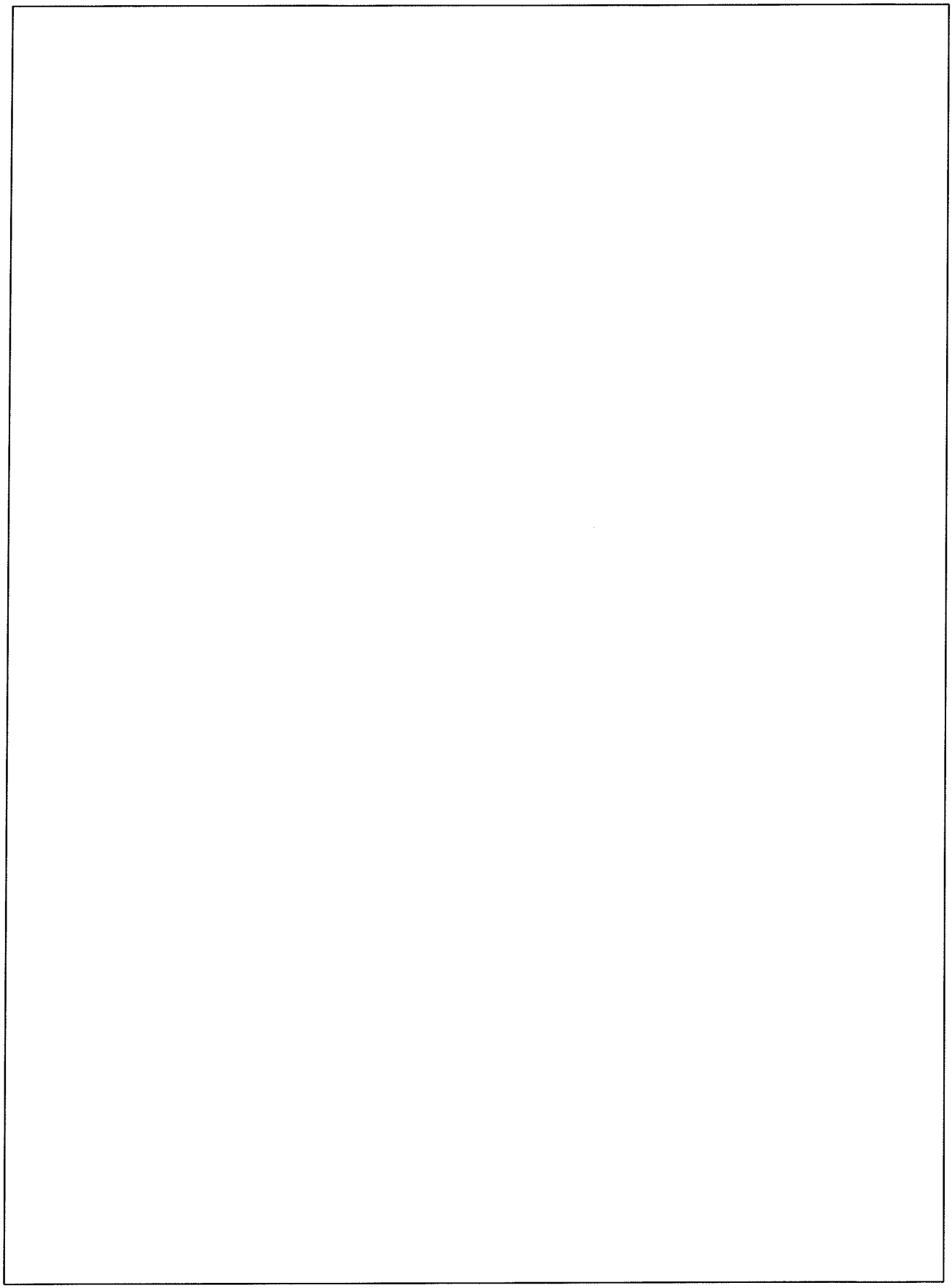


## B.3. Construction of the Subject Microorganism

### *B.3.a. Construction strategy.*







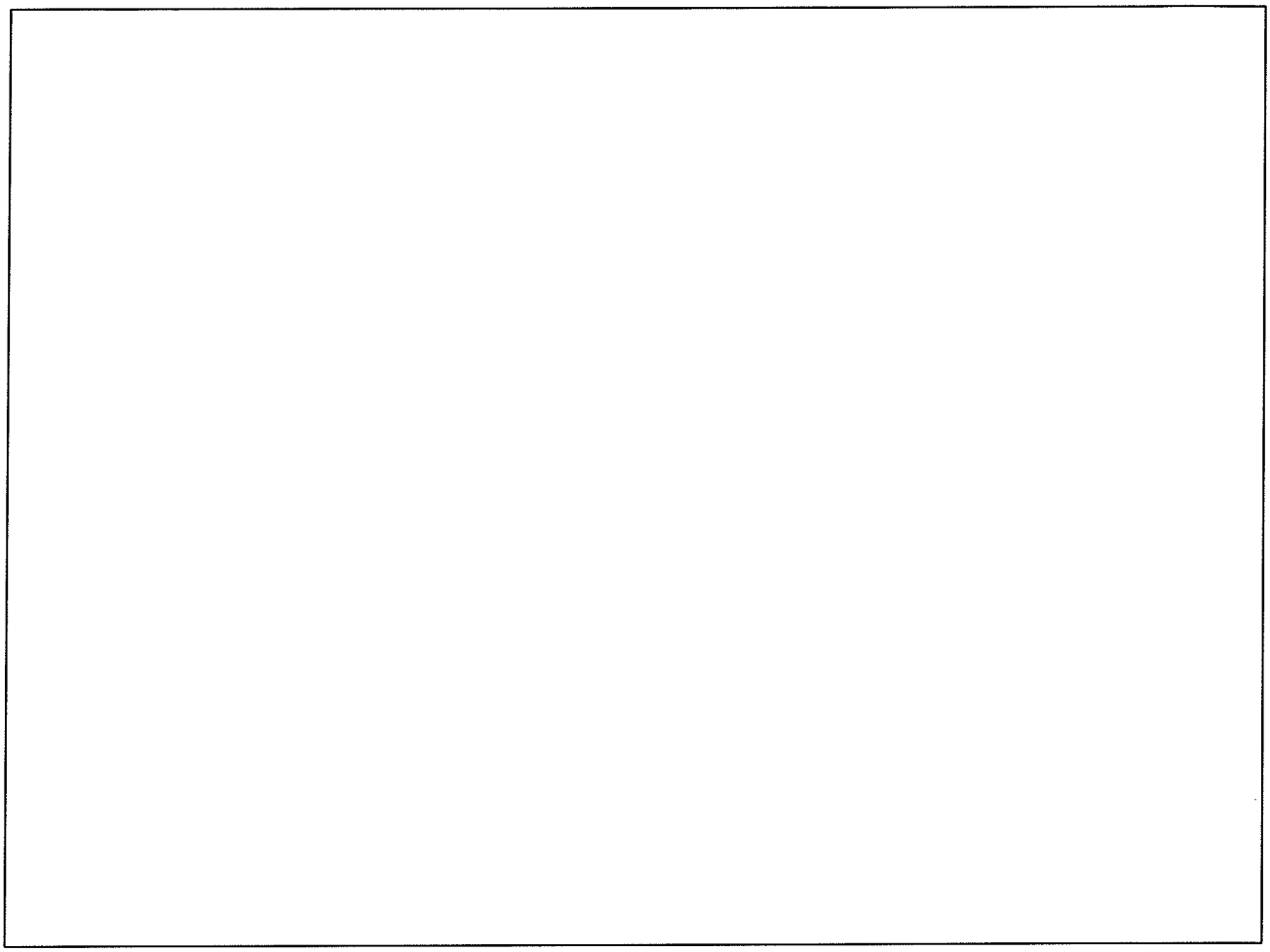


**Figure B-2. Flowchart of Strain Construction**

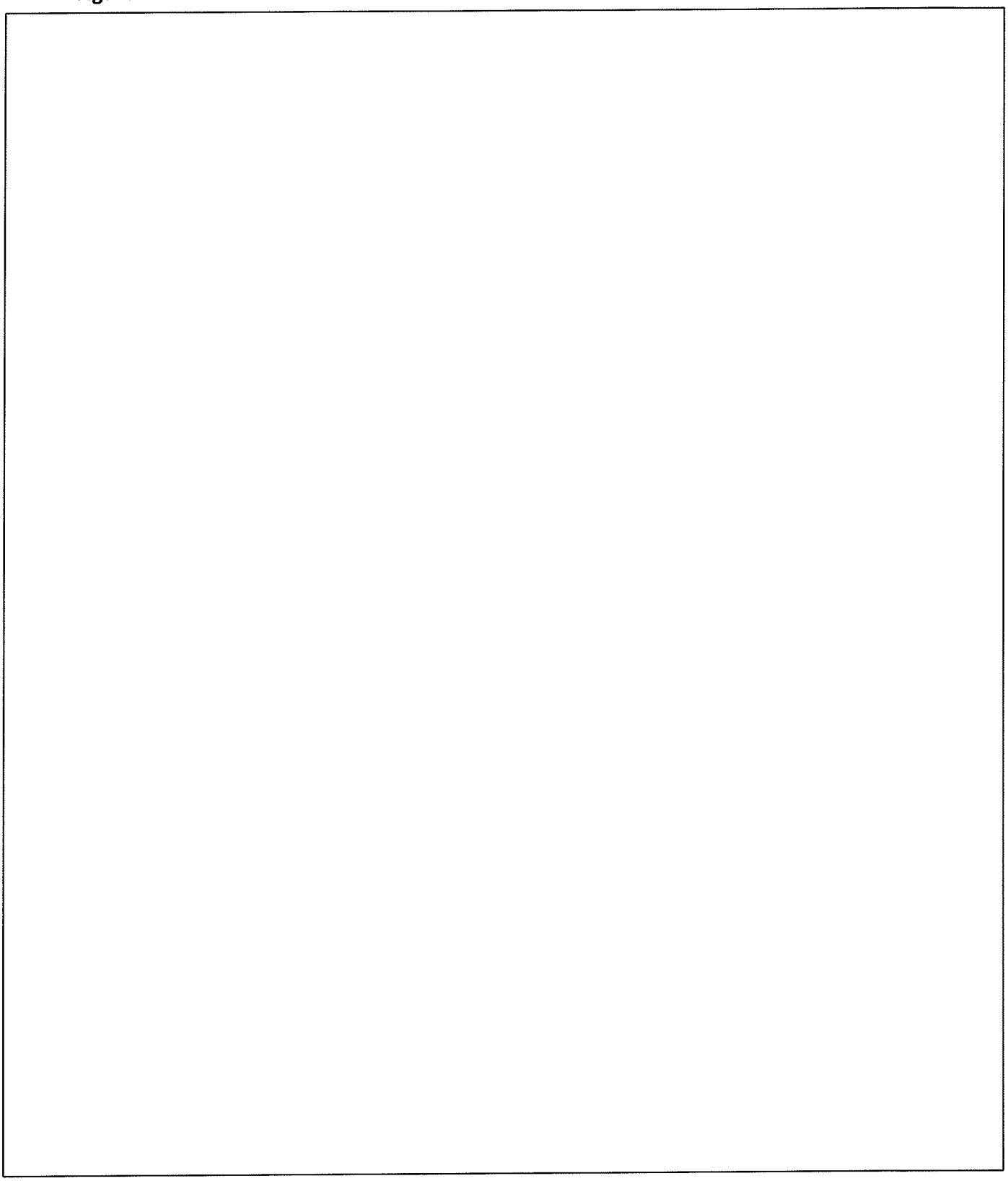


**Figure B-2. Flowchart of Strain Construction (continued)**

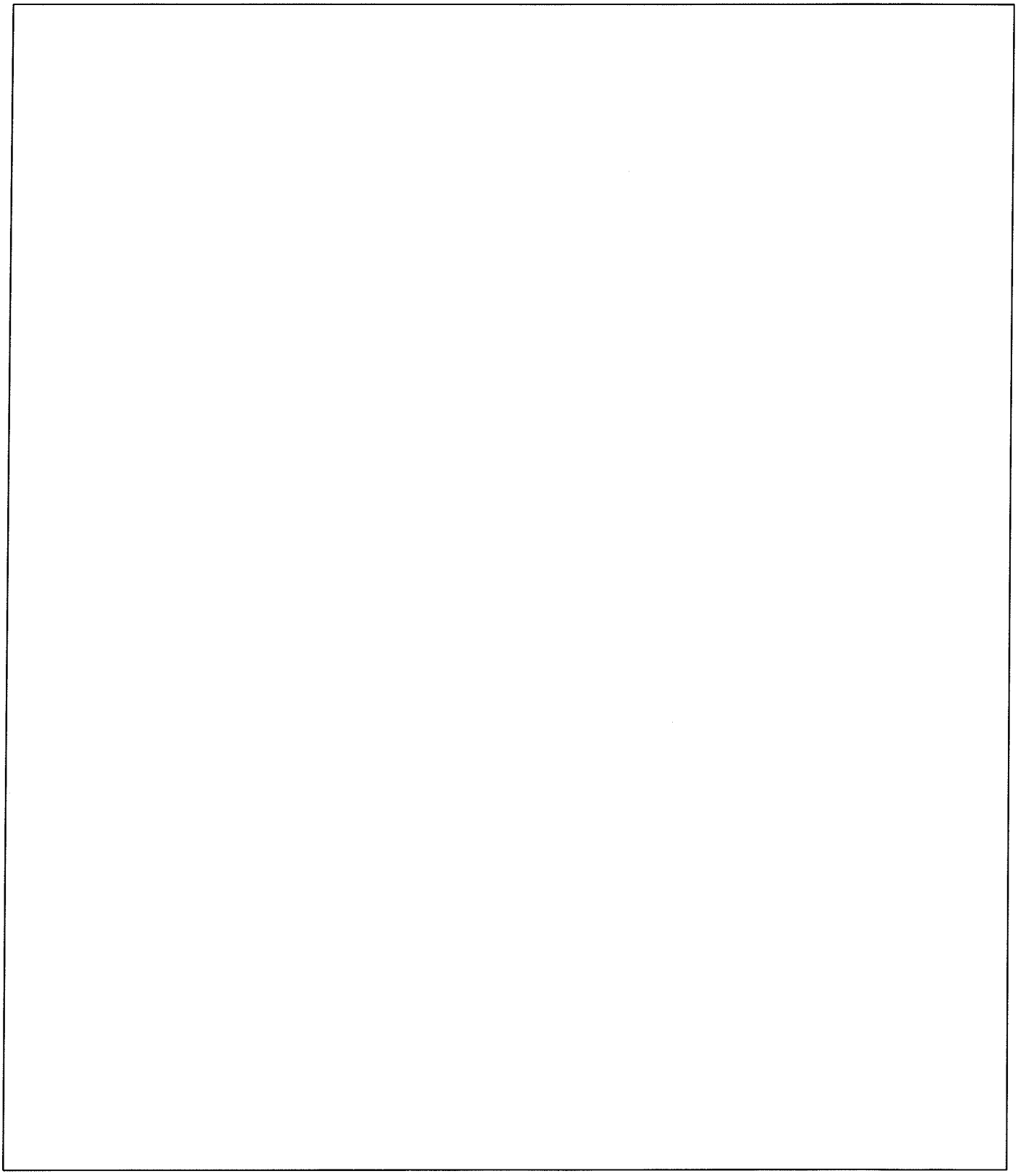
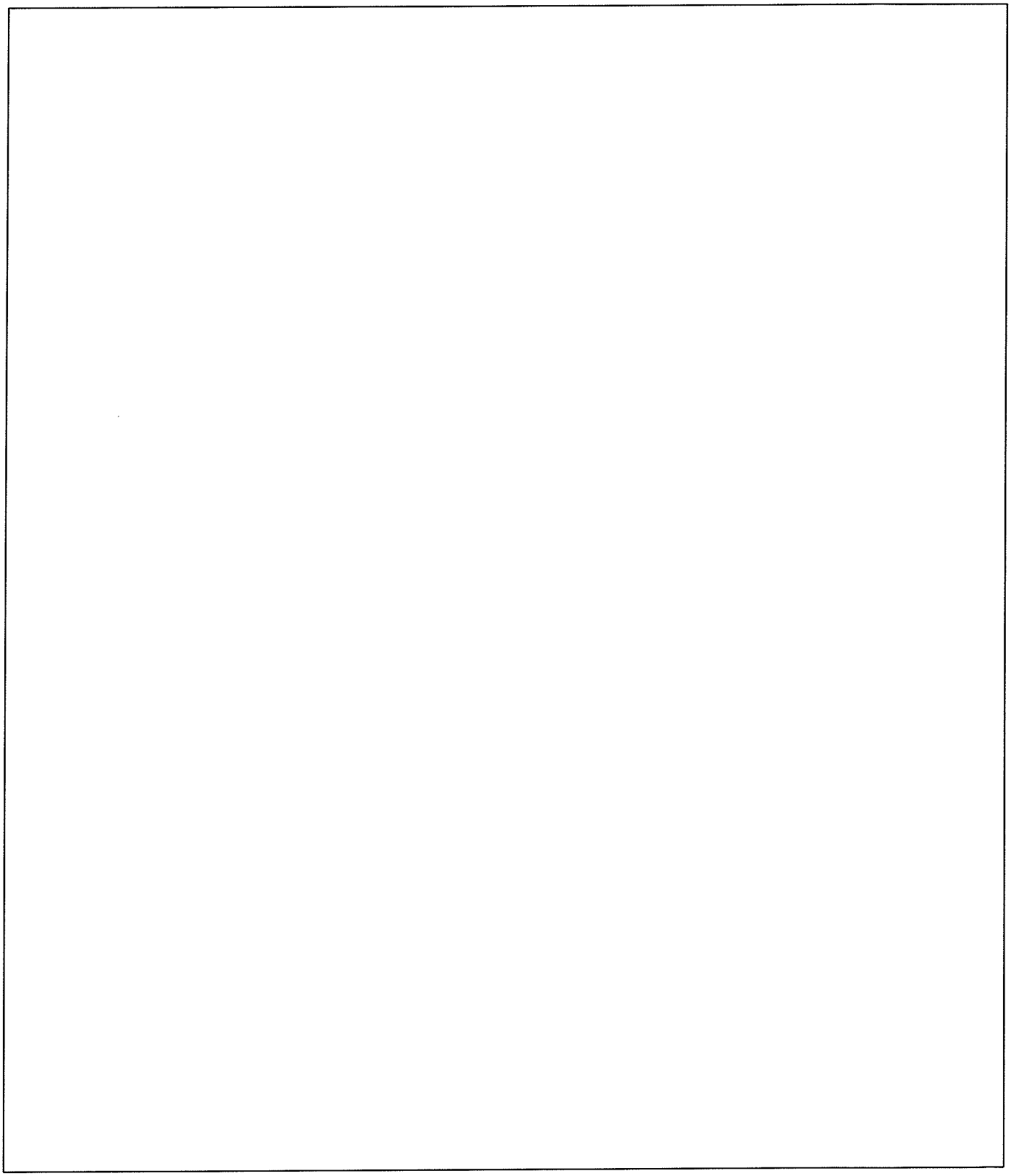
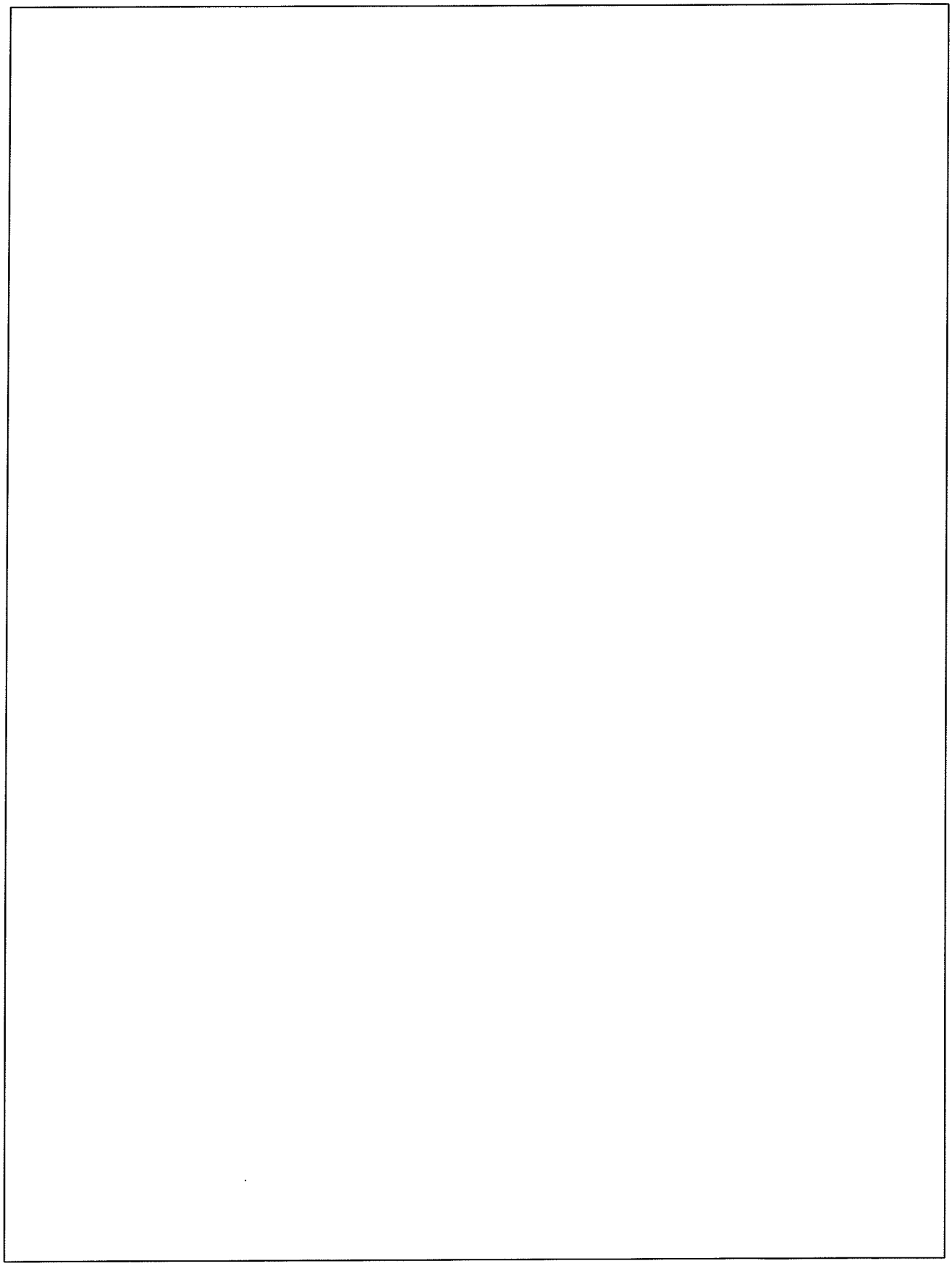
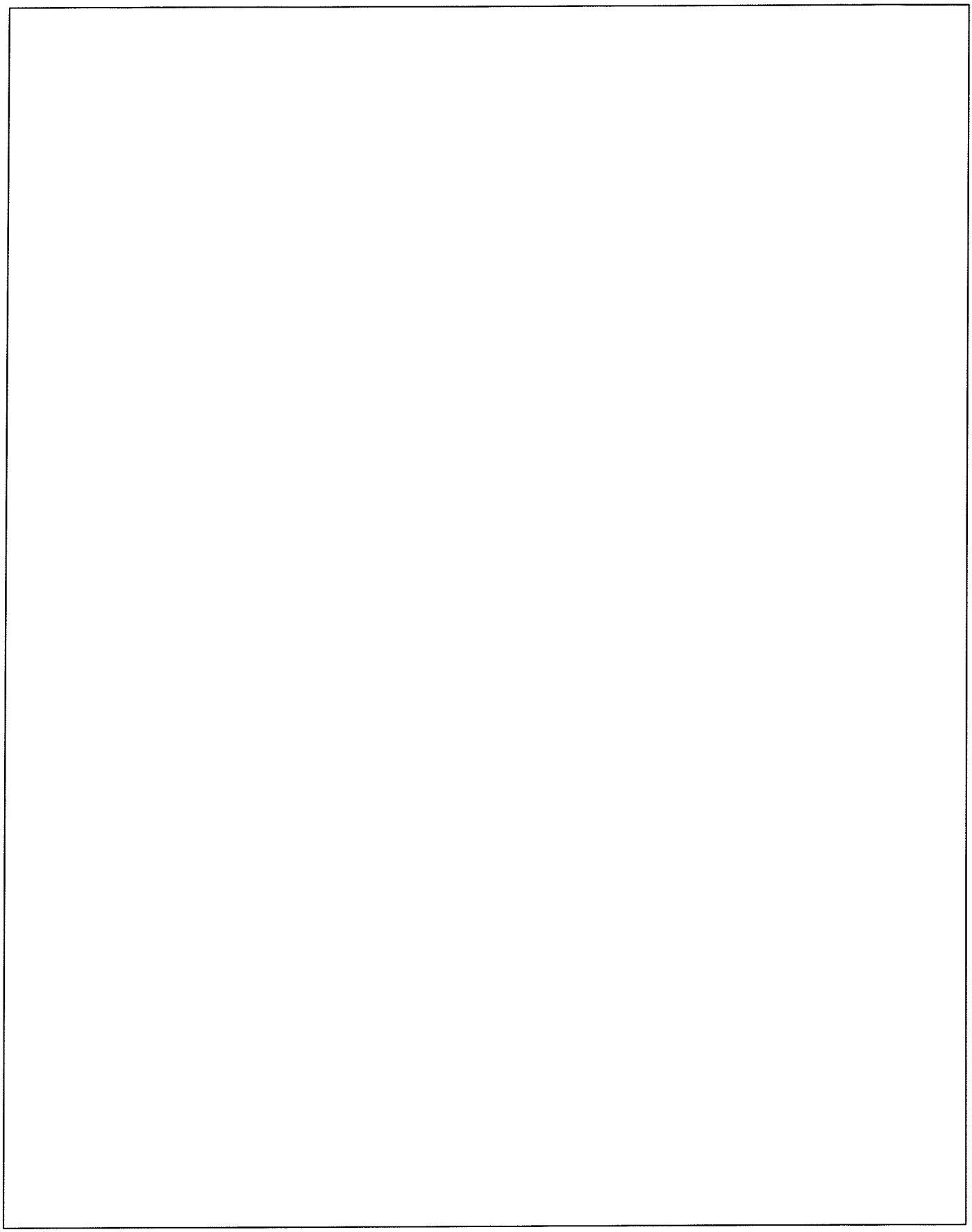
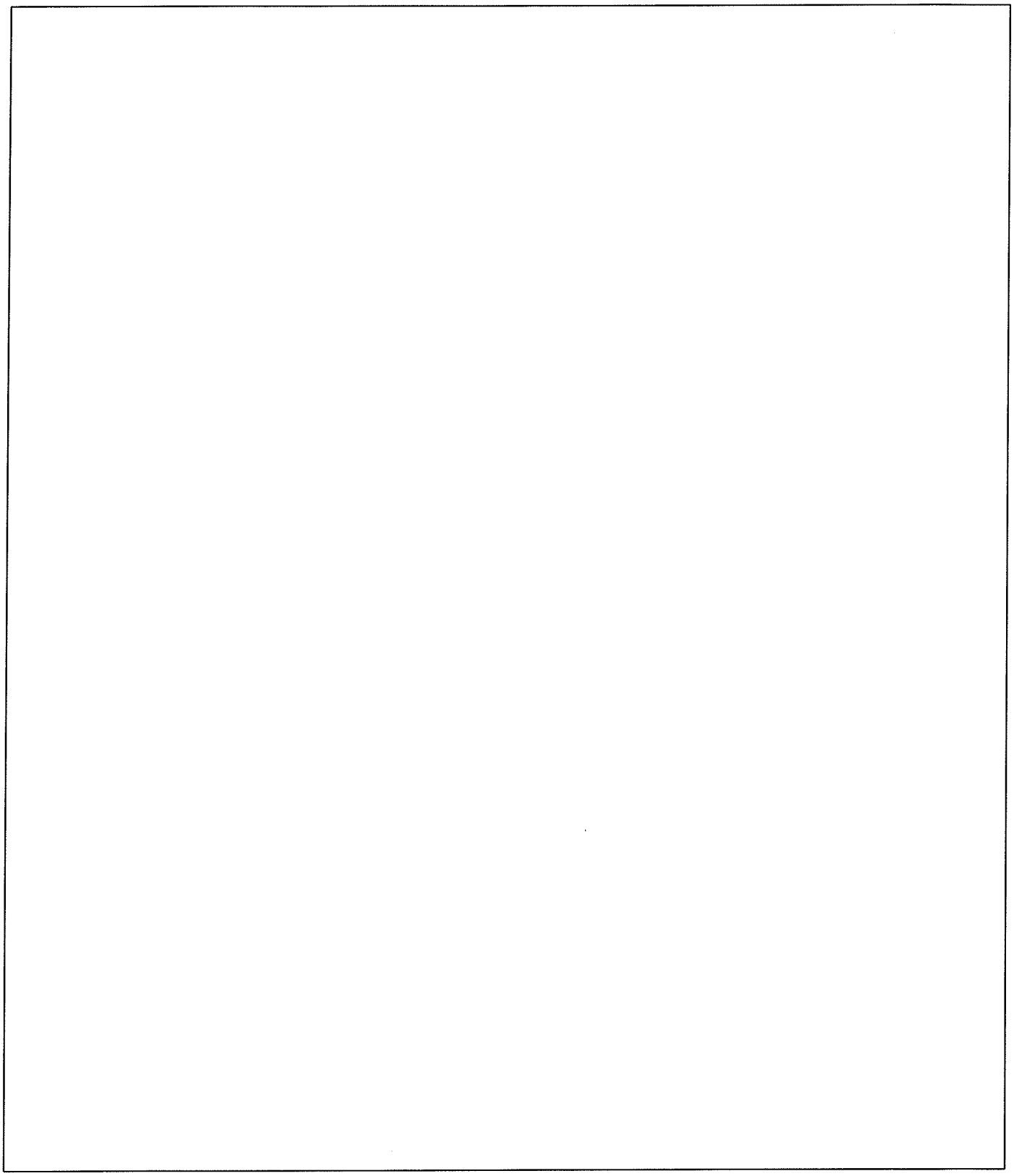


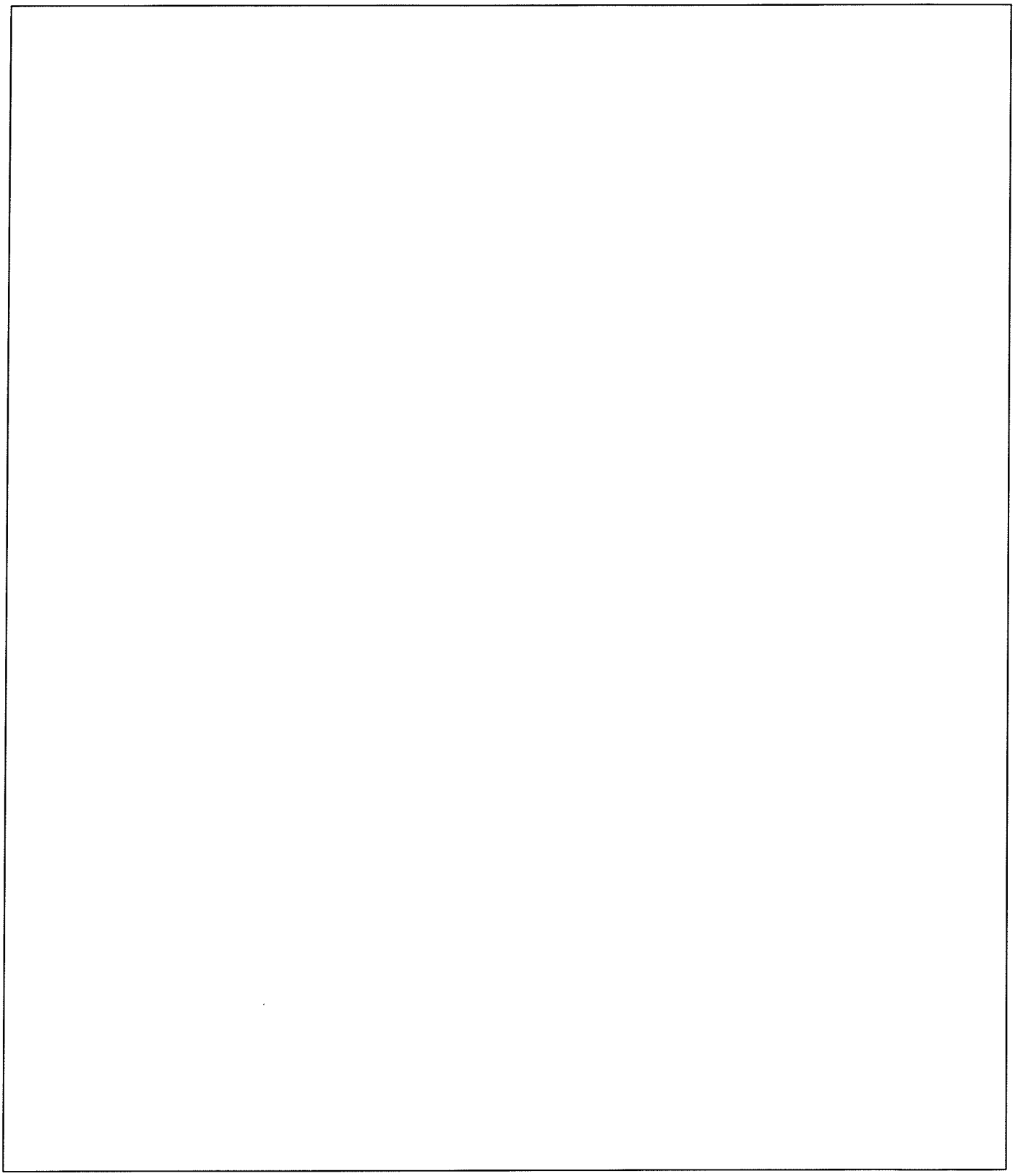
**Figure B-2. Flowchart of Strain Construction (continued)**

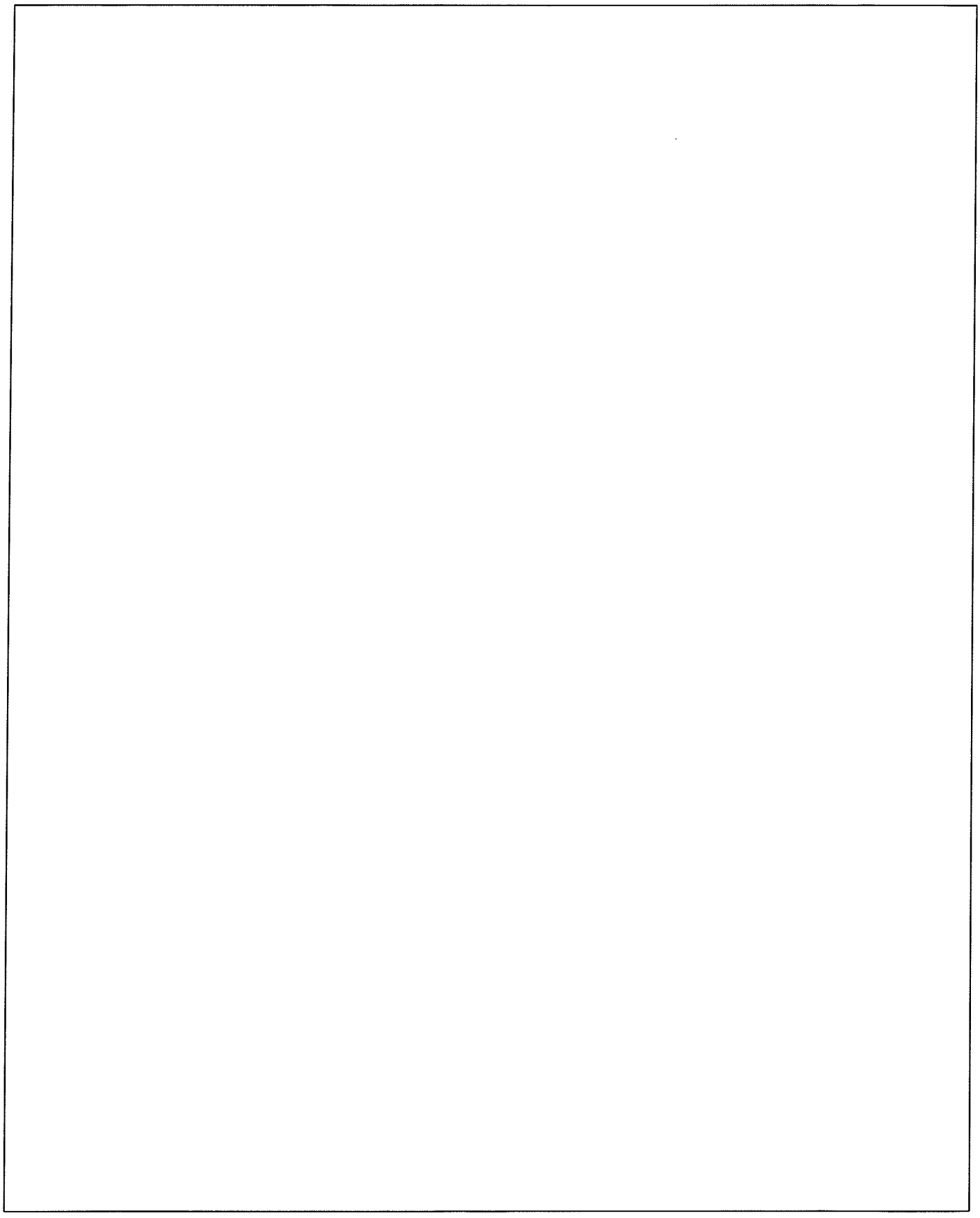












***B.3.b. Final recipient microorganism characterization***

*B.3.b.1. Prior modifications (deletions, additions).*

*B.3.b.2. Presence of plasmids.*

*B.3.b.3. Endogenous gene(s) homologous to the introduced DNA*

*B.3.b.4. Characterization of the insertion site for the introduced DNA*

***B.3.c. Prior submissions to EPA or other Federal Agencies.***

None.

***B.3.d. A key which contains full names for abbreviations used in the diagram.***

***B.3.e. Flowchart of Construction***

A flowchart showing the construction is found above as **Figure B-2**.

**B.4. Properties of the Subject Microorganism**

Much of the information requested in the Points to Consider document has been presented above. The following is additional information not discussed above.

***B.4.a. Methods and results used to verify the final construct.***

***B.4.b. Description of gene regulation and expression in the subject microorganism and the characteristics of the product encoded for the intended use.***

***B.4.c. Indication of whether the major gene product is extracellular or intracellular.***

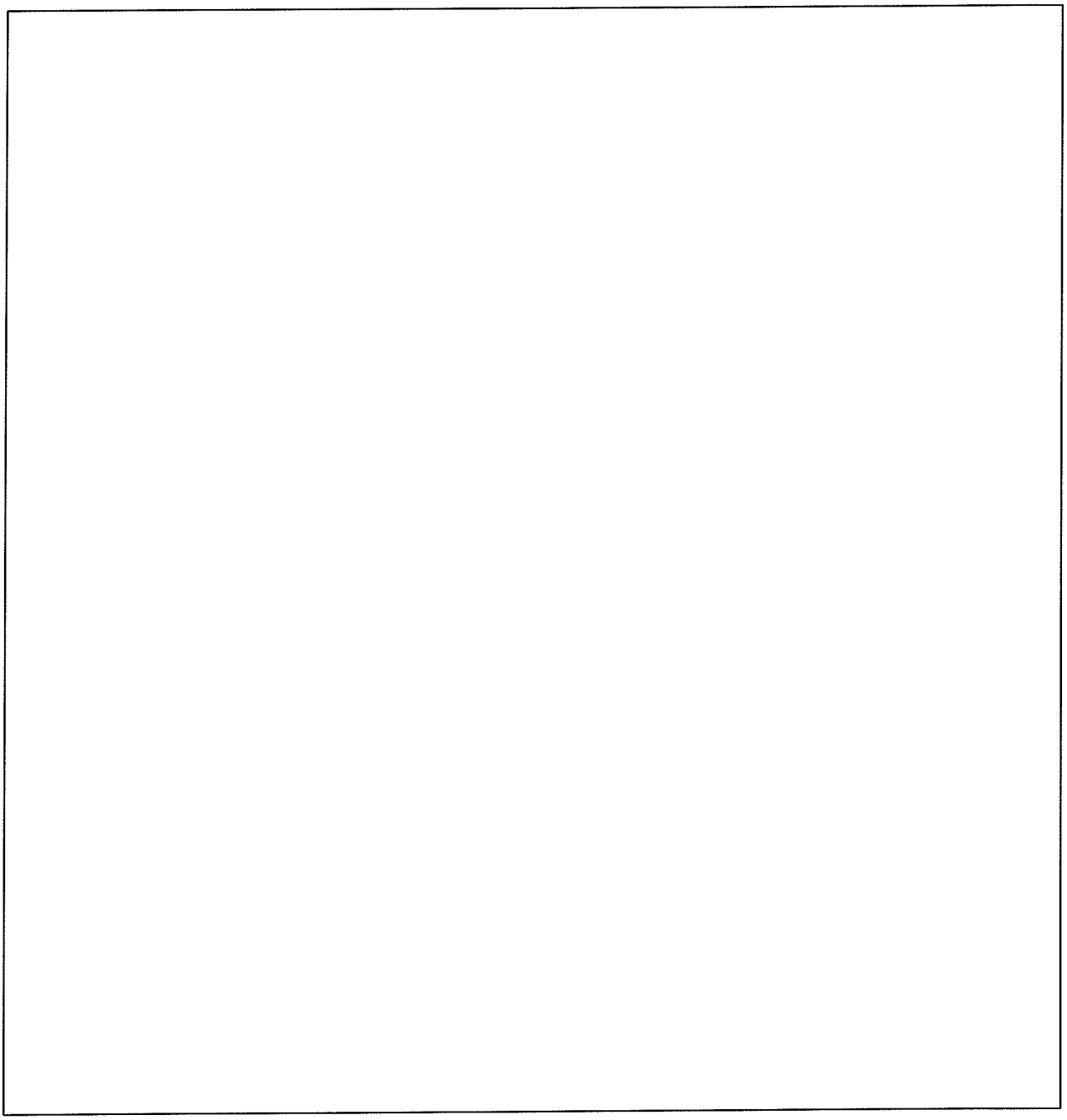
***B.4.d. Methods and results for determining stability of the introduced DNA***

***B.4.e. Growth characteristics in laboratory and environmental media***

***B.4.f. Factors limiting growth, survival, or reproduction***

***B.4.g. Subject microorganism antibiotic profile.***

**Figure B-6. Growth of MCAN strains compared to wild type.**



## C. POTENTIAL HUMAN HEALTH EFFECTS OF THE SUBJECT MICROORGANISM

### C.1. Pathogenicity of Subject Microorganism

*S. cerevisiae* has a long history of safe use in fermentations for numerous human and animal foods and beverages. It is widely accepted that *S. cerevisiae* poses no risks to human health or to the environment, and that strains of this organism are safe to use in fuel ethanol or chemical production processes.

EPA published a comprehensive risk assessment of *S. cerevisiae* in its 1997 rulemaking under TSCA in support of its decision to recommend this strain for the tiered exemptions (EPA,1997). This document stated:

> *Saccharomyces cerevisiae* has an extensive history of use in the area of food processing. Also known as Baker's Yeast or Brewer's Yeast, this organism has been used for centuries as leavening for bread and as a fermentor of alcoholic beverages. With a prolonged history of industrial applications, this yeast has been either the subject of or model for various studies in the principles of microbiology.

With regard to the possible health effects of this species, the risk assessment concluded:

> In conclusion, *S. cerevisiae* is a organism which has an extensive history of safe use. Despite considerable use of the organism in research and the presence of *S. cerevisiae* in food, there are limited reports in the literature of its pathogenicity to humans or animals, and only in those cases where the human had a debilitating condition. Factors associated with the virulence of yeasts (i.e., phospholipases) indicate that this organism is nonpathogenic. The organism has not been shown to produce toxins to humans.

*S. cerevisiae* has also been the subject of numerous MCANs previously filed by other applicants and reviewed by EPA, including MCAN Numbers J11-0001, J12-0004, J12-0005, J12-0007, J13-0003 to -0006, J13-0007, J13-0008, J13-0010, J14-0001, and J14-0003 through -0006. According to EPA's website, all these MCANs were dropped from review, and Notices of Commencement have been filed for several of these MCANs. Several of these MCANs have reviewed literature published subsequent to EPA's 1997 risk assessment, which has continued to support the contention that *S. cerevisiae* has no pathogenic or other adverse effects on human health.

### C.2. Toxicity and Immunological Effects of Subject Microorganism or Its Products

According to EPA (1997):

> There have been no reports of isolates of *S. cerevisiae* that produce toxins against either humans or animals. However, *S. cerevisiae* has been shown to produce toxins against other yeasts. These toxins, termed "killer toxins", are proteins or glycoproteins produced by a range of yeasts. The yeasts have been genetically modified to alter activity and are used in industrial settings as a means of controlling contamination of fermentation systems by other yeasts.

## D. PREDICTED ENVIRONMENTAL EFFECTS AND FATE OF SUBJECT MICROORGANISM

### D.1. Ecological Effects

The potential ecological effects of *S. cereivisiae* have been assessed in a number of previous risk assessment documents, including EPA (1997), the prior MCANs referenced above (see in particular MCANs J14-0001 and J14-0003 through -0006), as well as risk assessment documents from Environment Canada (e.g. Environment Canada 2006). No significant adverse environmental effects attributable to *S. cerevisiae* were identified in any of these prior risk assessments.

### D.2. Survival and Fate

#### *D.2.a. Natural habitats and geographical distribution of the recipient microorganism.*

According to EPA (1997):

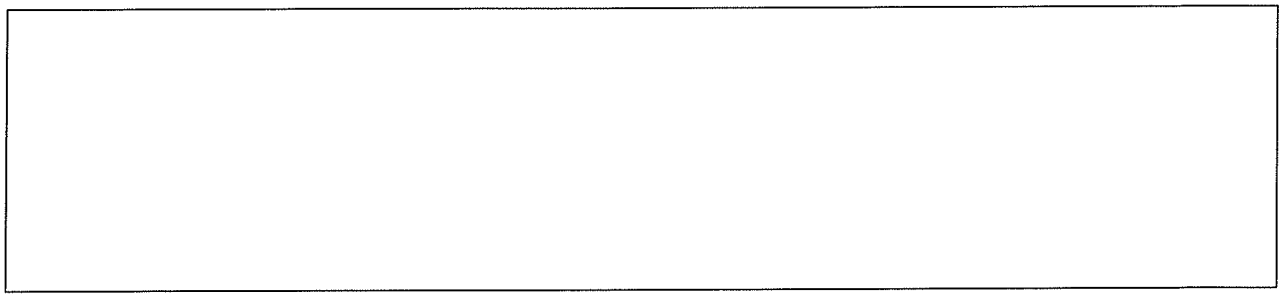
> *S. cerevisiae* is ubiquitous in nature. It has been recovered from a variety of sites under varying ecological conditions. The organism is used in a variety of industrial scenarios. *S. cerevisiae* is commonly recovered from a variety of fresh fruits and vegetables, generally those fruits with high levels of fermentable sugars. However, it is not listed as the causative agent of food spoilage for fruits and vegetables.

A more recent publication, Liti et al. (2006), reported the isolation of *S. cerevisiae* strains from each continent.

#### *D.2.b. Survival/persistence in environmental media e.g. soil, water, and/or air.*

EPA (1997) assessed the information known to date regarding survival and persistence of of S. cerevisiae in soil, water and air. The assessment noted that "soil is a natural habitat for *S. cerevisiae*, [so] it would be expected to survive well in soil", but that "worst case estimates do not suggest high levels of exposure of *S. cerevisiae* to either workers or the public resulting from normal fermentation operations".

#### *D.2.c. Method of detection and detection limits of microorganism in soil and/or water*



## E. PREDICTED PRODUCTION VOLUME, BYPRODUCTS, USE, AND CONSUMER EXPOSURE

### E.1. Information on Production Volume

It is anticipated that, during the first year of commercial use, the MCAN strains would be used at an ethanol facility having a [ ] gallon per year capacity. It is estimated that the maximum daily production volume of the MCAN strains would be [ ], and that the expected maximum production volume for the first year would be approximately [ ].

Over the first three year period, it is expected that the MCAN strains would be used in facilities having a total capacity of [ ] gallons of ethanol per year. The maximum daily production volume in any 12 month period during this time is expected to be [ ] and the expected maximum total production volume for any 12 month period would be approximately [ ].

### E.2. Information on Byproducts

[ ]

Due to the precise techniques employed to genetically modify *Saccharomyces cerevisiae* strains such as the MCAN strains, there is no reason to expect the production of undesirable products which could result from poorly controlled or characterized genetic modifications.

**E.3. Use Information and Consumer Exposure**

The end product that will be produced by the MCAN strains is fuel ethanol, which will be sold to blenders or distributors for blending into gasoline for use as motor vehicle fuel. There will be no live microorganisms found in the final commercial product. Therefore, it is expected that there will be no exposure of consumers to any of the MCAN strains.

## F. PREDICTED RELEASES DUE TO MANUFACTURING OF THE SUBJECT MICROORGANISM, AND WORKER AND CONSUMER EXPOSURES TO THE SUBJECT MICROORGANISM

### F.1. Industrial Sites for Use of the MCAN Strains

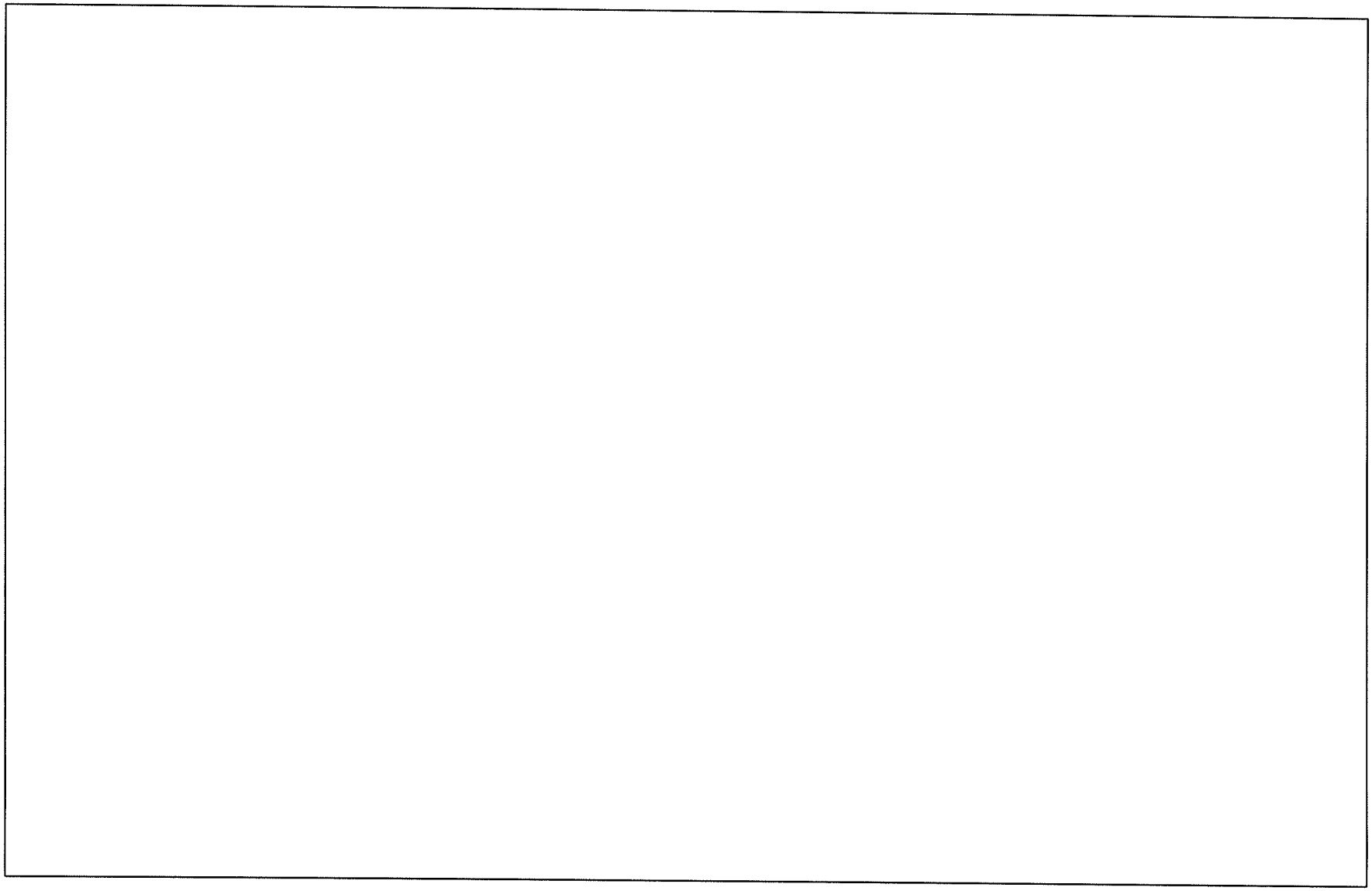
#### *F.1.a. Operation description*

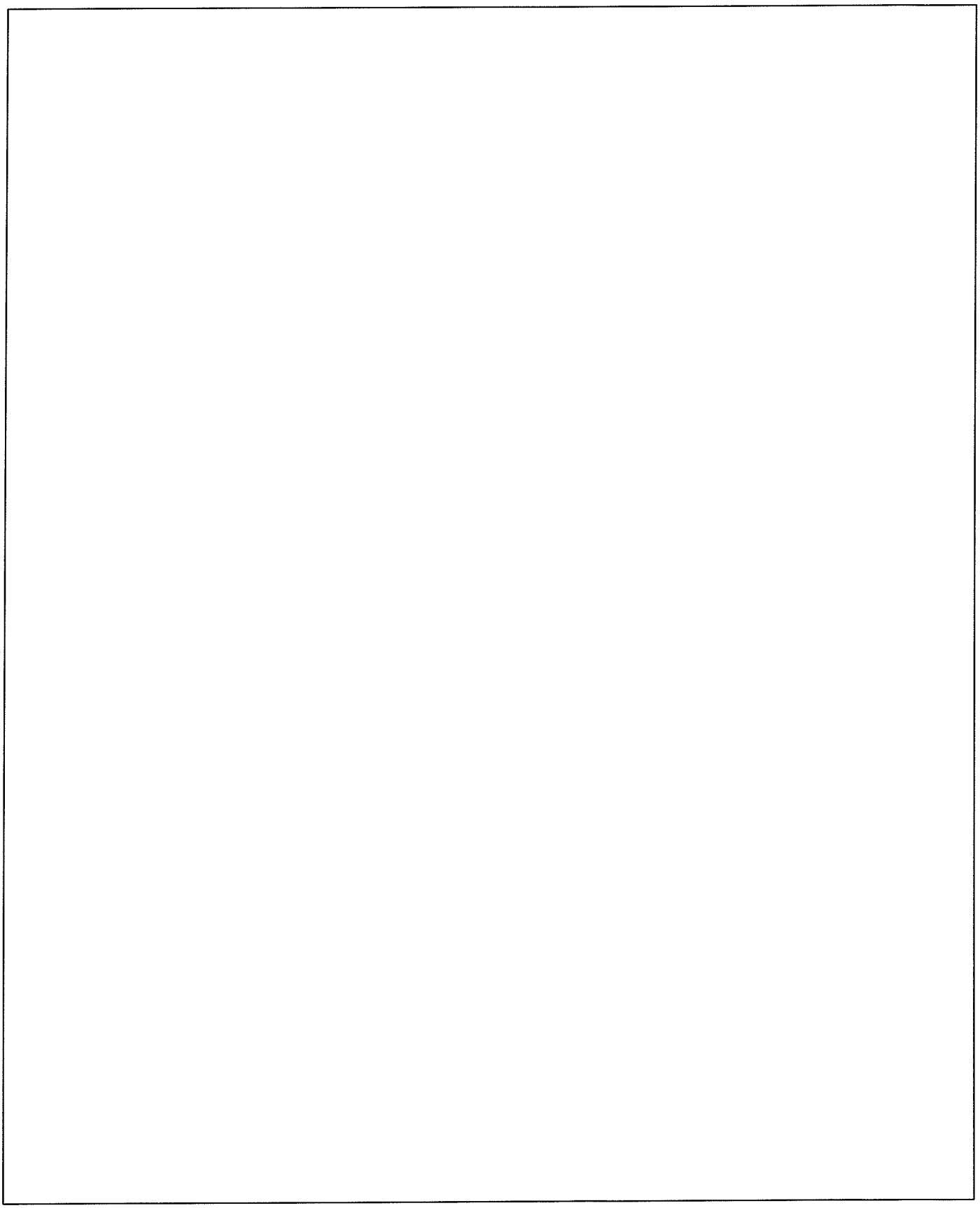
The MCAN strains will be used in fermentations using a variety of sugar feedstocks for the commercial production of ethanol. The MCAN strains can be used in any traditional manufacturing process where yeast strains are fermented to produce ethanol. A representative such process is described in Schell, et al. (2004) (**Attachment 7**).

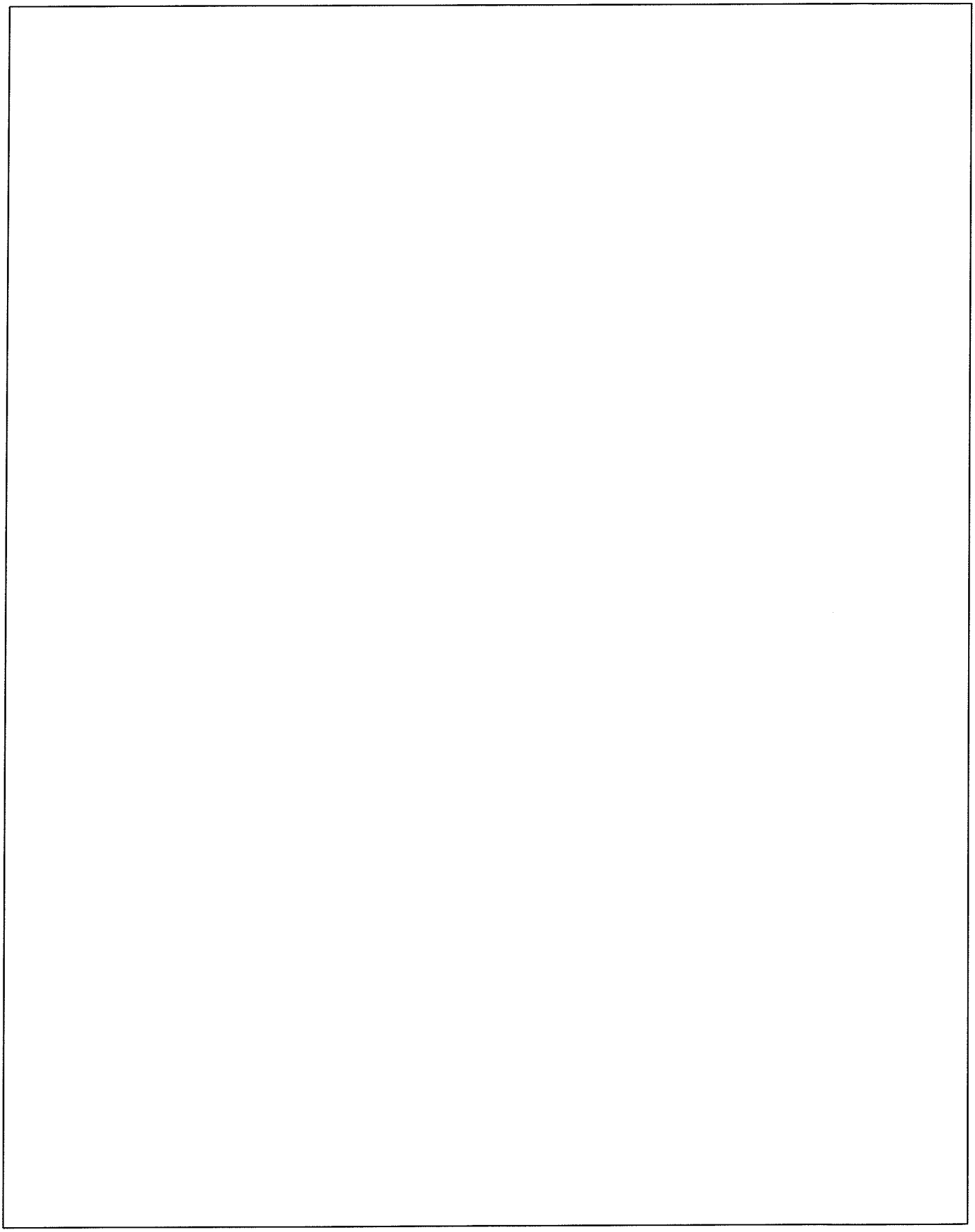
*F.1.a.1. Identity - identity of the site at which the operation will occur to include the name, site address and city, county, state and zip code.*

*F.1.a.2. Process description*

**Figure F-1. Process Flow Diagram.**







### *F.1.b. Occupational Exposure*

*F.1.b.1. Description of activities in which workers may be exposed to the recombinant microorganisms*

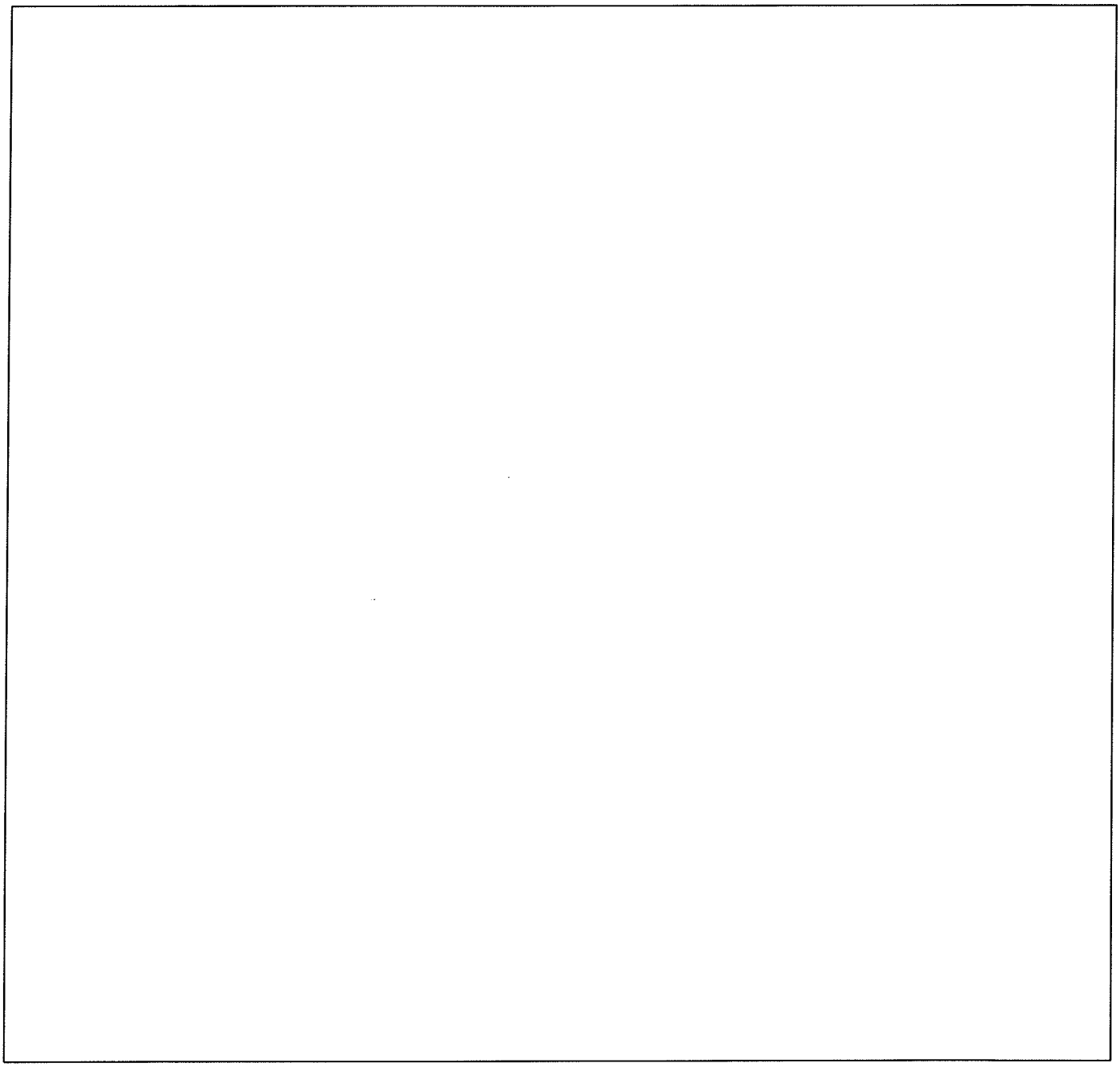
**Table F-1: Potential worker exposure to viable MCAN strains**

### *F.1.c. Environmental Release and Disposal*

*F.1.c.1. Estimates of release at each release point.*

This is shown in **Table F-2**.

*F.1.c.2. Control technologies at each release point.*



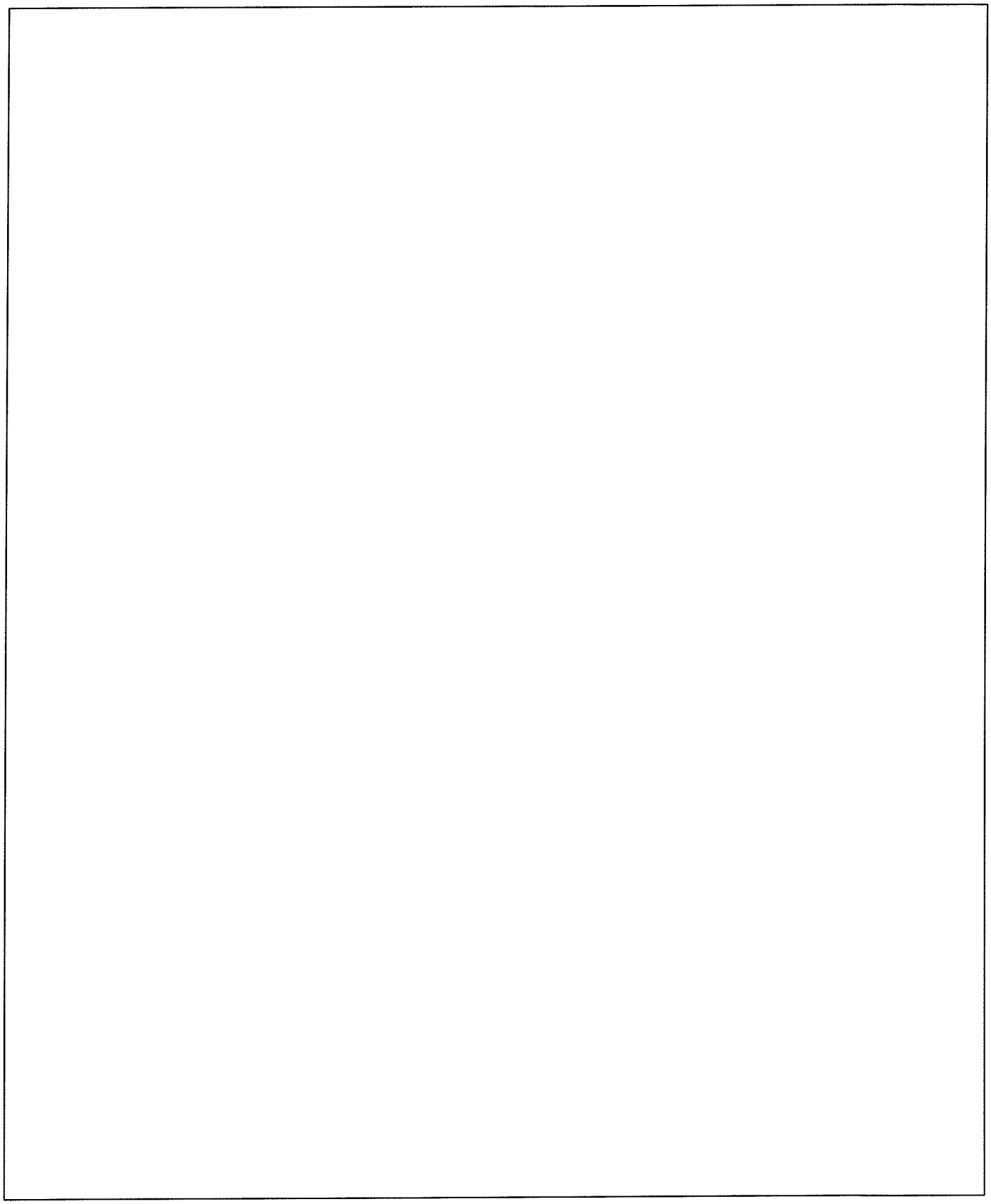


**Table F-2. Release points and daily amounts of new organism released into control technology and into the environment in ethanol production facility.**

Based on daily organism production estimated at

## G. REFERENCES

Berben, G., J. Dumont, V. Gilliquet, P.-A. Bolle, and F. Hilger. (1991) "The YDp plasmids: a uniform set of vectors bearing versatile gene disruption cassettes for Saccharomyces cerevisiae" Yeast 7, 475–477.

Environment Canada 2006, Risk Assessment Summary Conducted Pursuant to the New Substances Notification Regulations (Organisms) of the Canadian Environmental Protection Act, 1999; EAU-288: Saccharomyces cerevisiae strain ECMo01, https://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=AECC21AD-1

EPA, 1997. "Saccharomyces cerevisiae Final Risk Assessment, http://www.epa.gov/biotech_rule/pubs/fra/fra002.htm

Gietz, R.D., Schiestl, R.H., Willems, A.R. & Woods, R.A. (1995) "Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure" Yeast 11, 355-360.

Liti G., Barton D.B.H., Louis E.J. (2006) Sequence Diversity, Reproductive Isolation and Species Concepts in Saccharomyces. *Genetics*. 174(2):839-850. doi:10.1534/genetics.106.062166.

Rothstein, R. (1991) Targeting, Disruption, Replacement, and Allele Rescue: Integrative DNA Transformation in Yeast. Meth. Enzymol. 194, 281-301.

Schell, D.J.; Riley, C.J.; Dowe, N.; Farmer, J.; Ibsen, K.N.; Ruth, M.F.; Toon, S.T.; Lumpkin, R. (2004). "A Bioethanol Process Development Unit: Initial Operating Experiences and Results with a Corn Fiber Feedstock."*Bioresource Technology* (91); pp. 179-188.

Thompson, J.R., Register, E., Curotto, J., Kurtz, M. and Kelly, R. (1998) "An improved protocol for the preparation of yeast cells for transformation by electroporation" Yeast 14, 565-571.

Verduyn, C., Postma, E., Scheffers, W.A. & Van Dijken, J.P. (1992) "Effect of benzoic acid on metabolic fluxes in yeasts: a continuous-culture study on the regulation of respiration and alcoholic fermentation" Yeast 8, 501-517.